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Cardiorespiratory responses to low-level ozone exposure: The inDoor Ozone Study in childrEn (DOSE)

Jing Huang[a,1], Yi Song[b,1], Mengtian Chu[a], Wei Dong[a], Mark R. Miller[c], Miranda Loh[d], Junhui Xu[a], Di Yang[a], Rui Chi[a], Xuan Yang[a], Shaowei Wu[a], Xinbiao Guo[a,*], Furong Deng[a,*]

[a] Department of Occupational and Environmental Health Sciences, School of Public Health, Peking University, Beijing, China
[b] Institute of Child and Adolescent Health, School of Public Health, Peking University, Beijing, China
[c] University/BHF Centre for Cardiovascular Science, Queens Medical Research Institute, The University of Edinburgh, Edinburgh, UK
[d] Institute of Occupational Medicine, Research Avenue North, Riccarton, Edinburgh, UK

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ABSTRACT

Background: Indoor air pollution has emerged as a significant environmental and public health concern in recent years. However, evidence regarding the cardiorespiratory effects of indoor ozone is limited, and the underlying biological mechanisms are unclear, especially in children. Our study aimed to assess the cardiorespiratory responses to indoor ozone exposure in children.

Methods: A repeated-measure study was conducted in 46 middle-school children in Beijing, China. Real-time concentrations of ozone, along with co-pollutants including particulate matter (PM) and black carbon (BC), were monitored in classrooms from Monday to Friday. Three repeated health measurements of cardiorespiratory functions, including ambulatory electrocardiogram (ECG), blood pressure, fractional exhaled nitric oxide (FeNO) and lung function, were performed on each participant. Mixed-effect models were used to evaluate the effects of indoor ozone exposure.

Results: The mean (SD) indoor ozone concentration was 8.7 (6.6) ppb during the study period, which was largely below the current guideline and standards. However, even this low-level ozone exposure was associated with reduced cardiac autonomic function and increased heart rate (HR) in children. For instance, per interquartile range (IQR) increase in ozone at 2-hour moving average was associated with −7.8% (95% CI: −9.9%, −5.6%) reduction in standard deviation of all normal-to-normal intervals (SDNN), and 2.6% (95% CI: 1.6%, 3.6%) increment in HR. In addition, the associations were stronger at high BC levels (BC ≥ 3.7 μg/m^3). No significant associations were found for airway inflammation and pulmonary function.

Conclusions: Exposure to low-level indoor ozone that is not associated with respiratory effects was significantly related to disturbed cardiac autonomic function and increased HR in children, which suggested a possible mechanism through which ozone may affect cardiovascular health in children, and indicated more protective measures should be taken to alleviate the acute adverse effects of indoor ozone in this susceptible population.

Abbreviations: ATS/ERS, American Thoracic Society/European Respiratory Society; BC, black carbon; BMI, body mass index; CI, confidence intervals; CO_2, carbon dioxide; DBP, diastolic blood pressure; ECG, electrocardiogram; EPA, Environmental Protection Agency; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in one second; HF, high frequency; HR, heart rate; HRV, heart rate variability; IQR, interquartile range; LF, low frequency; LFHFR, the ratio of LF power to HF power; PEF, peak expiratory flow; PM, particulate matter; $PM_{2.5}$, fine particle; PM_{10}, inhalable particle; pNN50, the percentage of differences between adjacent normal-to-normal intervals larger than 50 ms; PP, pulse pressure; RH, relative humidity; rMSSD, the square root of the mean squared differences between adjacent normal-to-normal intervals; SBP, systolic blood pressure; SD, standard deviation; SDNN, standard deviation of all normal-to-normal intervals; WHO, World Health Organization

* Corresponding authors at: Department of Occupational and Environmental Health Sciences, Peking University School of Public Health, No. 38 Xueyuan Road, Beijing 100191, China.
E-mail addresses: guoxb@bjmu.edu.cn (X. Guo), lotus321321@126.com (F. Deng).
[1] These two authors contributed equally to this study.

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1. Introduction

Air pollution has emerged as a major environmental and public health concern worldwide (Cohen et al., 2017), with staggering levels of attributable morbidity and mortality (Dominici et al., 2006; Lepeule et al., 2012; Shah et al., 2013). As a key component of photochemical smog, ozone is a highly reactive and strongly oxidative secondary air pollutant (Bell et al., 2004). With the increase in vehicle numbers in large cities in China, air pollution mainly caused by conventional coal combustion has gradually transformed to both coal combustion and motor vehicle emissions (Kan et al., 2012). Thus, in addition to particulate matter (PM), ozone pollution has received increasing attention in recent years (Huang et al., 2018).

Epidemiological studies have shown that acute and long-term exposure to ambient ozone increases cardiorespiratory morbidity and mortality (Bell et al., 2004; Jerrett et al., 2009; Raza et al., 2014; Yin et al., 2017). However, there is far less evidence regarding the cardiorespiratory effects of ozone than regarding the effects of PM. Furthermore, most of the ozone studies focused on ambient exposure; the evidence of the cardiorespiratory effects of indoor ozone is limited, and the underlying biological mechanisms are unclear. Considering that most people spend > 80% of their time in indoor environments (Klepeis et al., 2001), a large fraction of total ozone exposure occurs indoors (Chen et al., 2012); thus, exploring the cardiorespiratory effects of indoor ozone exposure and the related biological mechanisms makes more sense for public health protection.

Previous studies that used environmentally controlled chamber to explore the cardiorespiratory responses and underlying mechanisms of indoor ozone exposure mainly focused on the acute impact of exposure (Arjomandi et al., 2015; Arjomandi et al., 2018; Barath et al., 2013; Devlin et al., 2012; Gong et al., 1998; Kahle et al., 2015; Rich et al., 2018). Nevertheless, the results were inconsistent. In addition, the exposure concentrations of ozone in the chamber studies mostly exceeded the current guideline and standards (U.S.EPA, 2015; WHO, 2006) and were higher than ozone levels in natural indoor environments (Day et al., 2017). Furthermore, the aforementioned studies were generally conducted in young healthy subjects or the elderly. Few data were available on children, who are more susceptible to the adverse health effects of air pollution considering that they are still growing and developing (Morgenstern et al., 2008).

The limited studies on ozone exposure and children mostly focused on ambient ozone exposure and merely explored respiratory effects, such as impacts on lung function or asthma (Angelis et al., 2017; Dai et al., 2018; Gent et al., 2003; Gold et al., 1999; Lin et al., 2008; McConnell et al., 2002; Nickmilder et al., 2007). The cardiorespiratory effects of indoor ozone exposure in children have seldom been reported until now.

Therefore, whether natural indoor environment ozone exposure at a relatively low level affects the cardiorespiratory health of children is an important scientific issue that deserves to be explored. Thus, we conducted the inDoor Ozone Study in childrEn (DOSE), which is a repeated-measure study to investigate the cardiorespiratory responses to indoor ozone exposure in school children. There are three main points: the natural indoor environment ozone exposure in classrooms, the vulnerable population (children), and both the respiratory and cardiovascular effects on this population. The results will provide evidence for health protection associated with indoor ozone exposure in children and will guide regulatory standard setting for indoor ozone levels.

2. Materials and methods

2.1. Study design and participants

This research was designed as a repeated-measure panel study. The study was conducted in a middle school in the suburb of Beijing from December 2017 to March 2018. Real-time concentrations of ozone, along with co-pollutants, including PM and black carbon (BC), were monitored in the classrooms during school time from Monday to Friday. Three repeated health measurements of cardiorespiratory functions, including ambulatory electrocardiogram (ECG), blood pressure, fractional exhaled nitric oxide (FeNO) and lung function, were performed on each child. The following criteria were used to recruit participants: under 14 years of age; living in Beijing for more than two consecutive years; not suffering from any health conditions; having no history of asthma or thoracic surgery; living in school dormitories from Monday to Friday. During the study period, the participants were instructed to stay within the classrooms as much as possible. A self-administered activity questionnaire was given to each participant during the study period. The participants were told to record the time and place when they went outside, such as for a lunch break or to visit the toilet.

The study protocol was registered on clinicaltrial.gov (NCT03319056) and approved by the Review Board of Peking University Health Science Center, which conforms to the Declaration of Helsinki. Before inclusion, written informed consent was provided by all participants and their guardians, and the participants could withdraw from the study at any time.

2.2. Exposure measurements

All exposure measurement devices were installed at the height of the breathing zone (approximately 1.2 m) at the same position in each classroom. Measurements started at 7:00 am and ended at 17:00 pm from Monday to Friday. Ozone concentrations were measured by a real-time monitor (Aeroqual Series 500; Aeroqual, New Zealand). Co-pollutants, including fine particle ($PM_{2.5}$), inhalable particle (PM_{10}), BC and carbon dioxide (CO_2), were measured simultaneously. In addition, noise level and meteorological factors, including temperature and relative humidity (RH), were also determined. The instruments used for measurements were as follows: size-fractionated PM (Model Handheld PC3016; GrayWolf Inc., USA), BC (microAeth Model AE51; Magee Scientific, Berkeley, CA, USA), CO_2 (Model HCZY-1; Tianjianhuayi Inc., Beijing, China), noise (Model ASV5910; Hangzhouaihua Inc., Hangzhou, China), real-time temperature and RH (Model WSZY-1B; Tianjianhuayi Inc., Beijing, China).

2.3. Health effects measurements

Health parameters were measured by trained investigators on Monday, Wednesday and Friday during the study. Ambulatory ECG monitoring, including heart rate variability (HRV), heart rate (HR) and ST-segment elevations, began at 8:00 am and ended at 15:00–16:00 pm, which is consistent with school time. Blood pressure, FeNO and spirometry measurements were conducted at 15:00–17:00 pm each time. To avoid possible variation between different investigators, the same investigator ran the same tests throughout the study whenever possible.

2.3.1. ECG monitoring

Ambulatory ECG monitoring was conducted using a 12-channel Holter recorder (model MGY-H12; DM Software Inc., USA) that was positioned on the participants using a standard protocol. The participants were instructed not to take any food or drink (e.g., coffee, wine, tea) that may affect HRV, HR or ST-segment and to avoid high intensity exercise on the day of and the day before health measurements. Participants were instructed to wear the Holter recorders for 7–8 h, during which they were told to stay indoors as much as possible and to record their activities in the formatted diaries. Further details and data processing procedures have been documented in our previous work (Pan et al., 2018).

2.3.2. Blood pressure measurement

Following at least 10 min of rest, upper arm blood pressure was measured three times using an automated oscillometric monitor (HEM-

7052; Omron Healthcare Co. Ltd., Japan) with a minimum 3-minute interval. The averages of the blood pressure values (from the second to the last measurement) within a 5-mmHg range of difference were calculated and recorded as the final outcomes.

2.3.3. FeNO and spirometry measurements

FeNO was measured by the NIOX VERO® machine (Aerocrine AB, Solna, Sweden) following standardized procedures (American Thoracic Society and European Respiratory Society, 2005). Participants were asked to refrain from exercise, food and drink for 1 h before measurement. After FeNO measurement, spirometry measurements, including forced expiratory volume in 1 s (FEV_1) and peak expiratory flow (PEF), were measured by a portable PEF meter (Model 2110; Vitalograph Ltd., UK) following the American Thoracic Society/European Respiratory Society (ATS/ERS) recommendations (Laszlo, 2006). For these two indicators, two to five measurements were conducted for each participant at each timepoint. When the relative difference of two measurements was < 10%, the better result of the two measurements was recorded for final analysis.

2.4. Statistical analysis

Mixed-effect models including a random intercept for each subject to account for correlations in repeated measurements were used to explore the effects of ozone on cardiorespiratory health parameters. All exposure measurements were recorded as 5-min segments in accordance with the HRV indices, and calculated as 1-hour average for ST-segment elevations and 8-hour average for daily health measurements. Health parameters were $\log_{10}$-transformed to improve the normality and stabilize the variance due to skewed distributions, except ST-segment elevations, among which there were zero values.

The following covariates were chosen a priori for the analysis, including gender, age, body mass index (BMI), hour of day, day of week, temperature, RH, CO_2 and noise. Long-term trend including day-of-measurement (the count of the day that the measurement was conducted over the whole study period), was also added in the model as the fixed-effect term.

The model was as follows:

$$Y_{it} = b_0 + u_i + b_1x_1 + ...+b_px_p + \beta \text{ exposure} + \varepsilon_{it}$$

where Y_{it} is the logarithm of the health measurement in subject i at time t, b_0 is the overall intercept, u_i is the specific random intercept for the subject i, x_1-x_p are covariates, b_1-b_p are the regression coefficients for x_1-x_p, β is the regression coefficient for ozone exposure, and ε_{it} is the error for subject i at time t. Single-pollutant models were used to estimate the main effects of ozone exposure, while two-pollutant models were used to examine the stability of the relationships.

Percent change with 95% confidence intervals (CI) in $\log_{10}$-transformed health measurements and value changes of ST-segment elevation associated with per interquartile range (IQR) increase in different moving averages of ozone were calculated as $[10^{(\beta \times \text{IQR})}\text{-}1] \times 100\%$, and 95% CI as$\{10^{[\text{IQR} \times (\beta \pm 1.96 \times SE)]}\text{-}1\} \times 100\%$, where β and SE were the estimated regression coefficient and the standard error, respectively (Wu et al., 2010).

The "nlme" package for R software was used for all analyses (Version 3.1.2). Statistical significance was defined as two-sided $p < 0.05$.

3. Results

3.1. Participants' characteristics

As shown in Table 1, 46 participants completed the whole study, while 2 withdrew due to school transfers. There were 24 (52%) boys and 22 (48%) girls. The ages ranged from 11 to 14 years old, with an average of 12.4 years and a standard deviation (SD) of 0.8 years. The average BMI was 18.8 among the participants.

Table 1
Demographic characteristics of the study participants.

Characteristics	Value
Number	46
Male (%)	24(52)
Female (%)	22(48)
Age, years	
Mean ± SD	12.4 ± 0.8
Range	11–14
BMI, kg/m^2	
Mean ± SD	18.8 ± 3.4
Range	14.2–33.5

Abbreviation: SD, standard deviation; BMI, body mass index.

Table 2
Descriptive information of indoor exposure measurements.

Variables	N[a]	Mean ± SD	Median	Range	IQR
Ozone, ppb	3097	8.7 ± 6.6	8.8	0–15.8	10.9
$PM_{2.5}$, μg/m^3	3097	75.4 ± 53.8	30.0	5.2–181.0	27.6
PM_{10}, μg/m^3	3097	563.9 ± 498.3	410.9	15.4–1072.5	500.4
BC, μg/m^3	3097	4.4 ± 3.3	3.7	0.7–9.5	4.0
RH, %	3127	51.5 ± 9.1	51.3	29.8–76.6	11.8
Temperature, °C	3127	15.2 ± 4.5	14.1	6.0–23.7	7.6
Noise, dB	3127	69.7 ± 8.7	70.4	52.5–90.6	12.8
CO_2, ppm	3127	1306.2 ± 521.6	1331.5	267.0–2871.0	762.8

Abbreviations: SD, standard deviation; $PM_{2.5}$, fine particle; PM_{10}, inhalable particle; BC, black carbon; RH, relative humidity; IQR, interquartile range.
[a] Observations after excluding all missing values and abnormalities.

3.2. Description of exposure measurements

Table 2 shows the descriptive statistics for concentrations of ozone, $PM_{2.5}$, PM_{10}, BC, RH, temperature and noise. The mean (SD) concentration of indoor ozone was 8.7 (6.6) ppb. Ozone was highly correlated with $PM_{2.5}$ and PM_{10} but not with BC (Supplemental materials, Table S1). In addition, the 1-hour average and 8-hour average of the variables were presented (Supplemental materials, Table S2).

3.3. Description of health measurements

Table 3 shows the descriptive statistics for health measurements among the participants during the whole study. Cardiovascular measurements in the children, including HRV indices, HR, ST-segment elevations and blood pressure, and respiratory measurements, including average levels of FeNO, FEV_1 and PEF, are presented. According to the self-reported activity diaries, all participants spent > 80% of their time in the classroom during the exposure monitoring period (data not shown).

3.4. Cardiovascular responses to indoor ozone exposure

Fig. 1 shows the estimated percent changes in the HRV/HR indices per IQR increase in ozone at different moving averages. Fig. 1A is the results for the single-pollutant models. As shown, almost all the HRV/HR indices, including the standard deviation of all normal-to-normal intervals (SDNN), the square root of the mean squared differences between adjacent normal-to-normal intervals (rMSSD), the percentage of differences between adjacent normal-to-normal intervals larger than 50 ms (pNN50), the low frequency (LF) and high frequency (HF), the ratio of LF power to HF power (LFHFR) and HR were significantly altered at different moving averages. The greatest changes of the indices were mostly found at 2-hour moving average. For example, SDNN and

Table 3
Descriptive information of health measurements.

Variables	N[a]	Mean ± SD
SDNN, ms	10,837	65.7 ± 23.3
rMSSD, ms	10,837	37.3 ± 16.2
pNN50, ms	10,837	17.4 ± 13.8
TP, ms^2	10,837	4041.2 ± 3287.2
LF, ms^2	10,837	966.8 ± 656.6
HF, ms^2	10,837	381.6 ± 355.6
LFHFR	10,837	3.9 ± 3.2
HR, min^{-1}	10,837	90.3 ± 12.9
II_ST, mV	889	0.1 ± 0.1
V2_ST, mV	889	0.3 ± 0.2
V5_ST, mV	889	0.1 ± 0.1
SBP, mmHg	133	105.4 ± 7.3
DBP, mmHg	133	64.2 ± 6.0
PP, mmHg	133	41.2 ± 5.6
FeNO, ppb	133	17.2 ± 7.2
FEV_1, L	133	2.2 ± 0.5
PEF, L/min	133	342.6 ± 79.4

Abbreviations: SDNN, standard deviation of all normal-to-normal intervals; rMSSD, the square root of the mean squared differences between adjacent normal-to-normal intervals; pNN50, the percentage of differences between adjacent normal-to-normal intervals larger than 50 ms; TP, total power; LF, low frequency; HF, high frequency; LFHFR, the ratio of LF power to HF power; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1 s; PFE, peak expiratory flow.

[a] Observations after excluding all missing values and abnormalities.

LF and HF were decreased by −7.8% (95% CI: −9.9%, −5.6%) and − 6.6% (95% CI: −11.6%, −1.4%), respectively, per IQR increase in ozone. HR was significantly increased by 2.6% (95% CI: 1.6%, 3.6%).

Fig. 1B–D shows the estimated percent changes in HRV/HR indices per IQR increase in ozone at different moving averages after adjusting for $PM_{2.5}$, PM_{10} and BC, respectively. As shown, the results did not substantially change after adjusting for $PM_{2.5}$ and PM_{10}, compared with the single-pollutant models. Though the PM_{10} concentrations were high in our study, most of them were coarse particles from the classroom floor, which was made of soil. The effects of PM_{10} on HRV/HR indices were small. However, the alterations of HRV/HR indices were greater in the two-pollutant models after adjusting for BC (Supplemental materials, Table S3). Thus, whether ozone and BC interacted with HRV/HR was explored by adding the multiplicative interaction term of ozone and BC in the model. The interaction term was statistically significant. The BC levels were dichotomized by the mean level as a cut-off point (3.7 $\mu g/m^3$), and the effects of ozone at high BC levels were compared with those at low BC levels. Significant changes in HRV/HR indices were observed in high BC group, while nonsignificant changes were found in the low BC group. For instance, the LF and HF were decreased by -25.5% (95% CI: −34.2%, −16.4%) and -18.1% (95% CI: −28.5%, −6.8%) per IQR increase in ozone at 2-hour moving average in the high BC group, but were slightly increased in the low BC group (Fig. 2).

Fig. 3 presents the estimated changes in the ST-segment elevations with ozone exposure. II_ST, V2_ST and V5_ST are three representative leads in ST-segment analyses. A 0.044 mV (95% CI: 0.005 mV, 0.081 mV) increase of II_ST elevation was marginally significantly associated with per IQR increase in ozone at 0-hour moving average, while nonsignificant associations were found at other moving averages. Blood pressure, including systolic blood pressure (SBP), diastolic blood pressure (DBP) and pulse pressure (PP), showed an increased trend with ozone exposure, although the trend was not significant (Fig. 4).

3.5. Respiratory responses to indoor ozone exposure

Nonsignificant results were observed for respiratory responses, but the greatest changes in those measurements were found at the 2-d lag average. The greatest decreases of FEV_1 and PEF were −7.4% (95% CI: −22.2%, 10.3%) and −10.0% (95% CI: −22.0%, 3.8%) at the 2-d lag average, respectively, and the greatest increase of FeNO was 22.9% (95% CI: −2.4%, 54.9%) at the 2-d lag average (Fig. 4).

4. Discussion

In this study, the indoor ozone concentrations were monitored in the classrooms, and children's cardiorespiratory functions, including HRV indices that reflect cardiac autonomic function, HR, ST-segment, blood pressure, airway inflammation and lung function, were measured. The principal finding is that indoor ozone exposure, at levels largely below the current guideline and standards (U.S.EPA, 2015; WHO, 2006), was significantly associated with reduced cardiac autonomic function and increased HR in children, though nonsignificant relationships with airway inflammation and lung function were found at this exposure level. Furthermore, the effects of indoor ozone exposure on the cardiovascular system were stronger at high BC concentrations, which indicated possible modification by BC.

Compared with previous studies that used human chamber to simulate indoor ozone exposure and explored the related acute cardiorespiratory responses (Arjomandi et al., 2015; Arjomandi et al., 2018; Barath et al., 2013; Devlin et al., 2012; Gong et al., 1998; Kahle et al., 2015; Rich et al., 2018), our study provided the effects of indoor ozone exposure in a more natural microenvironment. The mean ozone concentration in our study was only 8.7 ppb, which was much lower than that in the human chamber studies such as 200 ppb or even 300 ppb (Arjomandi et al., 2015; Barath et al., 2013). Furthermore, the results were inconsistent in human chamber studies when comparing different populations. For instance, a study found that exposure to 0, 100, and 200 ppb ozone in a random order for 4 h induced dose-dependent adverse changes in the frequency domains of HRV in 26 adults (10 with mild-asthma) (Arjomandi et al., 2015), while another study did not find an effect of a similar doze of ozone on HRV in healthy young men (Barath et al., 2013).

However, we found that indoor ozone exposure at a relatively low-level was significantly associated with reduced HRV in children in our study, which was reflected by decreased parasympathetic modulation (SDNN, rMSSD, pNN50, LF and HF) and increased sympathetic drive (LFHFR). HRV is a widely used non-invasive method to investigate cardiac autonomic function (Electrophysiology, 1996). The cardiac autonomic nervous system is responsible for regulating the electrical control of the heart, and decreased HRV has been reported to be associated with cardiac autonomic dysfunction in children (Baumann et al., 2018; Jarrin et al., 2015; van Biljon et al., 2018). Thus, the results of our study indicated a possible physiological mechanism by which indoor ozone exposure may affect cardiovascular health through disturbed cardiac autonomic function in children. In addition, the acute effects of ozone on HRV were found in children not suffering from any health conditions. Considering that HRV reduction has important clinical significance related to electrical instability and cardiac arrhythmias (Phadumdeo and Weinberg, 2018), decreased HRV after ozone exposure, if sustained, could potentially increase the risk of clinical cardiovascular events such as arrhythmias in individuals with pre-existing disease.

For cardiovascular effects, we also observed a significant HR increment associated with ozone exposure and a marginally significant association with II_ST elevation, which is a clinical indicator of ischemic burden (Fournier et al., 2013). Blood pressure showed an increased trend, though the trend was not significant.

As a strong oxidant, ozone may induce lipid peroxidation and the production of cyclooxygenase, which triggers neural receptors of the airway (Chen et al., 1999). Thus, the response of the children's respiratory function to indoor ozone exposure was also measured in the study. Our results showed that increased indoor ozone exposure was associated with an increase in FeNO and decreases in FEV_1 and PEF, although the associations did not reach the nominal level of statistical

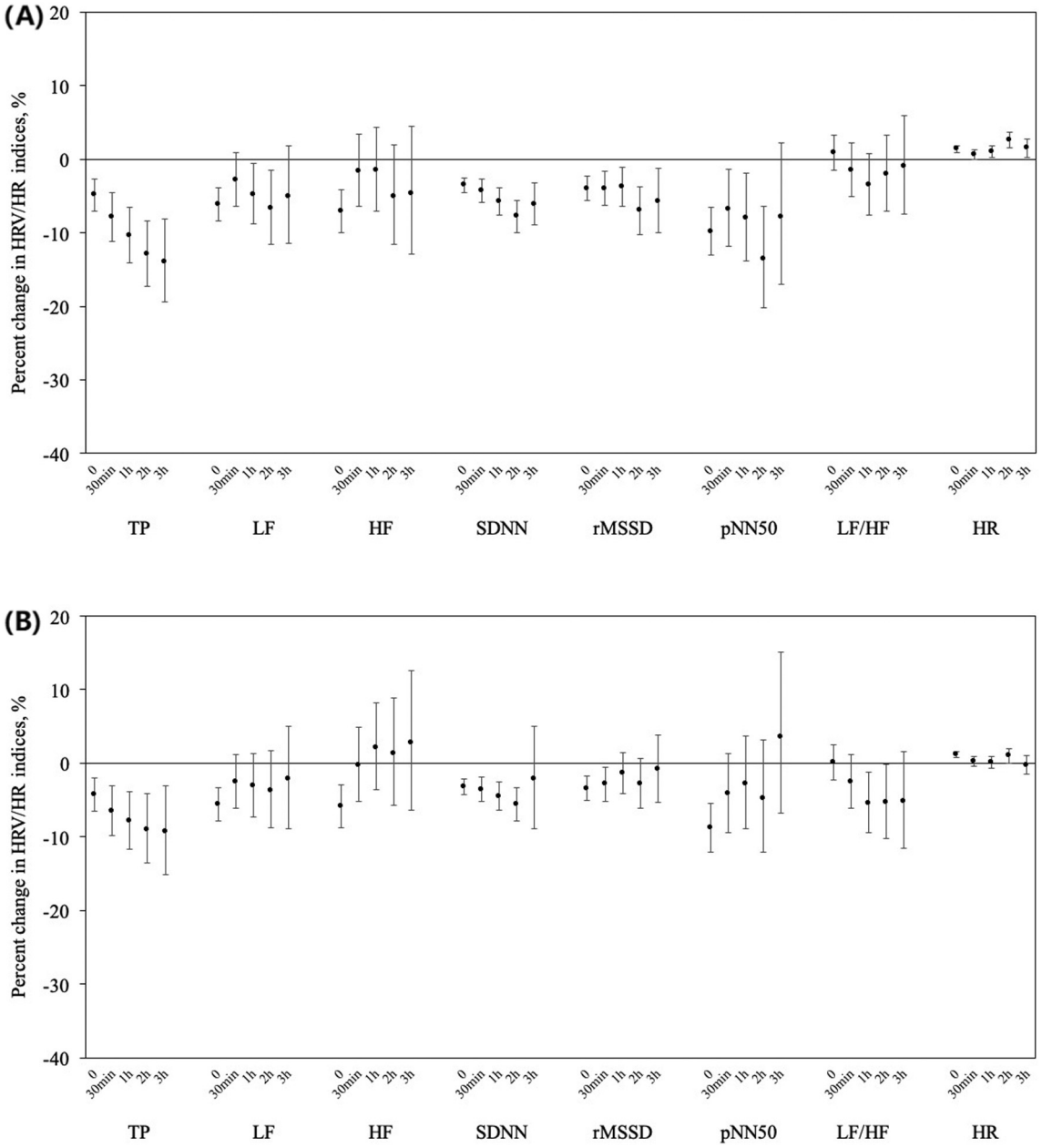

Fig. 1. Estimated percent changes with 95% confidence intervals in the HRV/HR indices per IQR increase in ozone at different moving averages. (A) Single pollutant; (B) adjusted for $PM_{2.5;}$ (C) adjusted for PM_{10}; (D) adjusted for BC.

significance.

FeNO is a reliable biomarker of airway inflammation that can be evaluated before spirometric changes occur (Sandrini et al., 2010). FeNO has also been used as an easy-to-measure and noninvasive marker of the effects of air pollution on the lower respiratory system (van Amsterdam et al., 2000). A previous study found a highly significant increase in FeNO related to ambient ozone exposure in children, and a threshold for this ozone-induced increase in FeNO estimated a benchmark dose of 110 μg/m^3 for 8 h, which was much higher than the exposure level in our study (Nickmilder et al., 2007).

Pulmonary function as an index of respiratory health effects of the lower airway has been documented in previous studies (Chen et al., 1999). Epidemiologic studies that investigated the acute pulmonary effects of ambient ozone exposure in children have shown negative associations between ozone exposure and pulmonary function. However, the ozone concentrations were very high in these studies. For instance, the peak hourly ozone concentration was 80 ppb in a study conducted in Taiwan (Chen et al., 1999), and the maximum daily average ozone concentration was > 100 ppb in a study conducted in Mexico City (Gold et al., 1999). In our study, the lung function of children decreased when exposed to indoor ozone, though not to a statistically significant degree. The result was similar to a study conducted on 47 school children in Greece, where the personal ozone concentrations were lower than 10 ppb (Angelis et al., 2017).

Integrating cardiovascular and respiratory responses, we found that exposure to low-level indoor ozone not associated with airway inflammation and lung function was significantly associated with cardiac autonomic function reduction and HR increment in children. The results indicated that cardiac autonomic function reflected by HRV indices and the HR level maybe more sensitive for capturing the acute effects of ozone exposure than respiratory outcomes. Previous studies also indicated disturbed cardiac autonomic function after ozone exposure (Devlin et al., 2012; Jia et al., 2011).

Therefore, it seems that the effects of ozone exposure on the

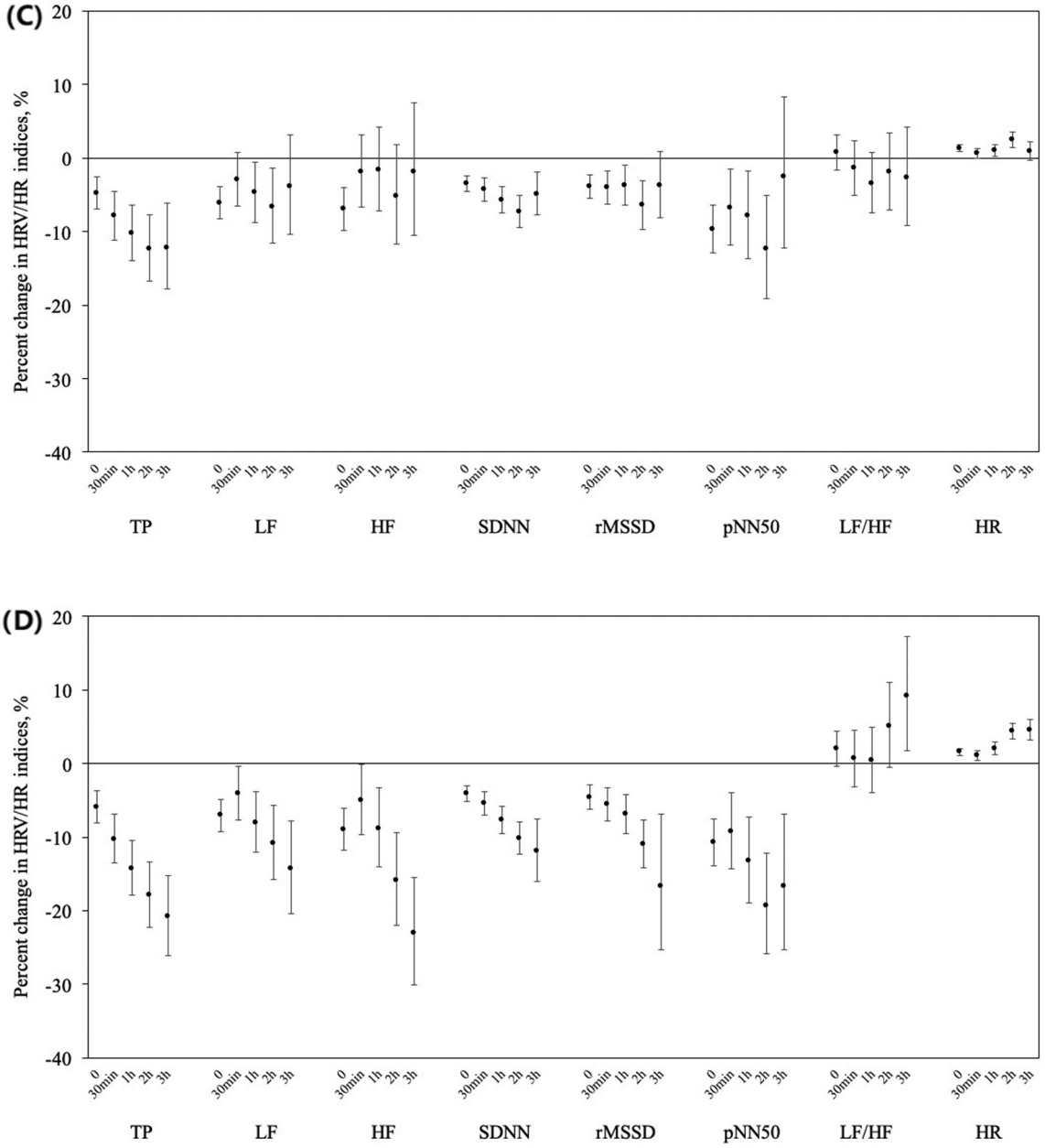

Fig. 1. (*continued*)

cardiovascular system may be independent of the effects on the respiratory system, and occur at a lower level than effects on the respiratory system. A recent study also found that 24-hour ozone exposure with a mean concentration < 10 ppb was associated with indicators of cardiovascular effects in healthy adults, while non-significant lung function impairment was found (Day et al., 2017). However, the current regulatory standards for ozone are mainly based on the respiratory effects observed in epidemiological studies and in environmental chamber exposure studies (Kim et al., 2011; Schelegle et al., 2009). Thus, the findings in our study indicated that future standards for ozone should take into consideration its associations with cardiovascular effects, which may occur at lower levels than can cause respiratory effects.

Nevertheless, more caution should be taken to interpret the significant associations of cardiac autonomic function indicators in our study. Considering that panel study design is not a causal inference method and the collinearity between the air pollutants, we could not distinguish the relative contributions of ozone exposure. In addition, as ozone in the indoor environment reacts quickly with various unsaturated organic compounds to produce highly reactive gaseous and condensed phase products (Weschler, 2006), this study could not determine whether the observed associations were associated with ozone itself or its reaction products. Furthermore, we only explored the acute effects of indoor ozone exposure on cardiorespiratory outcomes, the long-term impacts should be determined in future studies.

In this study, we found that the effects of ozone on cardiac autonomic function indicators and HR remained robust after adjusting for PM, and were stronger at high BC concentrations. This observation indicated possible modification by BC. BC has a large specific surface area and undergoes an aging process though which it can be oxidized by oxidants, such as ozone. There have been toxicological studies that showed ozone oxidized BC had more oxidative potential and higher cytotoxicity than BC alone (Chu et al., 2018; Gao et al., 2017). However, the biological mechanisms regarding the effects of ozone and BC on cardiac autonomic function and HR are not clear and still need to be explored by further studies.

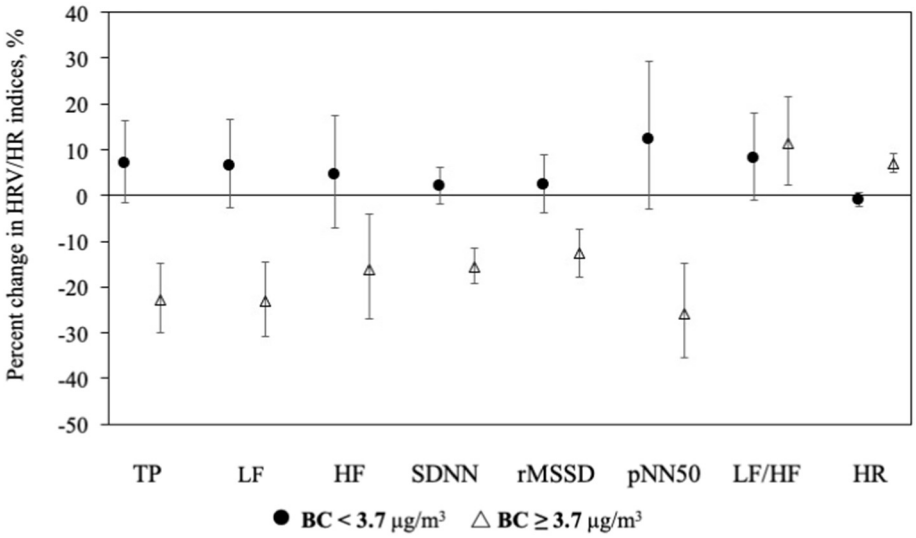

Fig. 2. Estimated percent changes with 95% confidence intervals in HRV/HR indices per IQR increase in ozone at 2-hour moving average. Solid circle: BC $<$ 3.7 μg/m^3; Empty triangle: BC $\geq$ 3.7 μg/m^3.

To the best of our knowledge, this is the first study to evaluate both the cardiovascular and respiratory responses to indoor ozone exposure in children who are more susceptible to air pollution. Furthermore, compared with the environmentally controlled chamber, this study presented a more natural indoor environment exposure. Low-level ozone exposure was associated with cardiac autonomic function reduction and HR increment in children, but not with respiratory indicators. The validity of this study was verified by strict quality control and statistical and biological plausibility analyses. Given that indoor air pollution has become a significant public health challenge and ozone pollution has received increasing attention in recent years, the study results will provide potential policy implications to minimize the adverse health effects of indoor ozone exposure in children.

Considering that indoor sources of ozone include outdoor ozone penetration as a main source, and that some devices and appliances designed for indoor use, including printers, photocopiers, electrostatic precipitators, etc. (Salonen et al., 2018), reduce the penetration of outdoor ozone, and limit the use of devices that emit ozone indoors could be effective methods to reduce indoor ozone pollution and related health risks.

However, our study has several limitations. First, night-time ozone exposure was not measured. However, most of the significant effects were found within 3 h, thus the effects of day-time ozone exposure were captured. In addition, the participants in our study stayed in the school dormitories during the study period, so the night-time ozone level may not vary substantially among the participants. Second, we only evaluated the acute cardiorespiratory impacts of indoor ozone exposure in a certain age group of children in this study. Considering that the effects of ozone may vary among different ages and timeframes (Dai et al., 2018; Devlin et al., 2012), replication of these findings is warranted in an independent sample in future studies, including among children of different age groups, and over various timeframes, such as the long term or different seasons.

5. Conclusions

Our study found that short-term indoor ozone exposure with a mean concentration $<$ 10 ppb was associated with disturbed cardiac autonomic function and increased HR in children, while nonsignificant changes in respiratory indicators were found at this level. The findings

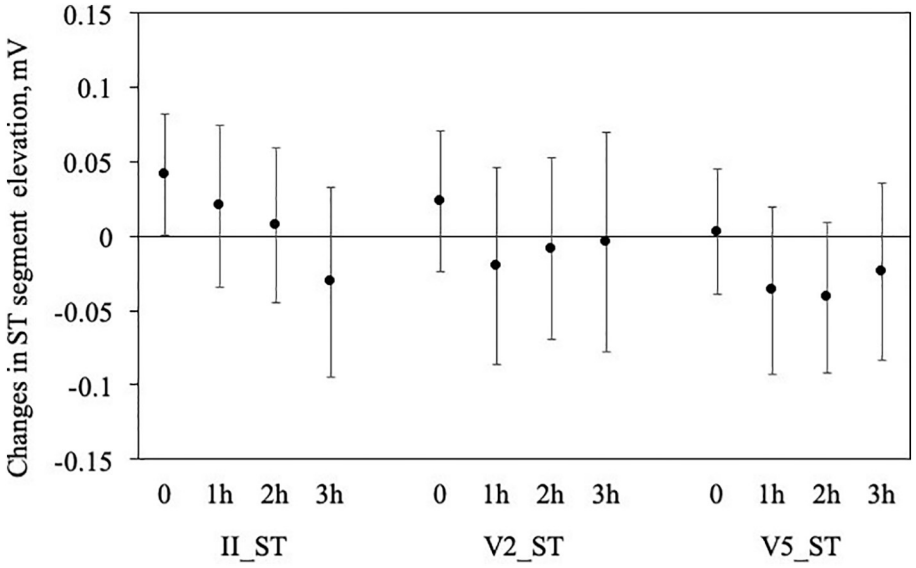

Fig. 3. Estimated changes with 95% confidence intervals in ST-segment elevations per IQR increase in ozone at different moving averages.

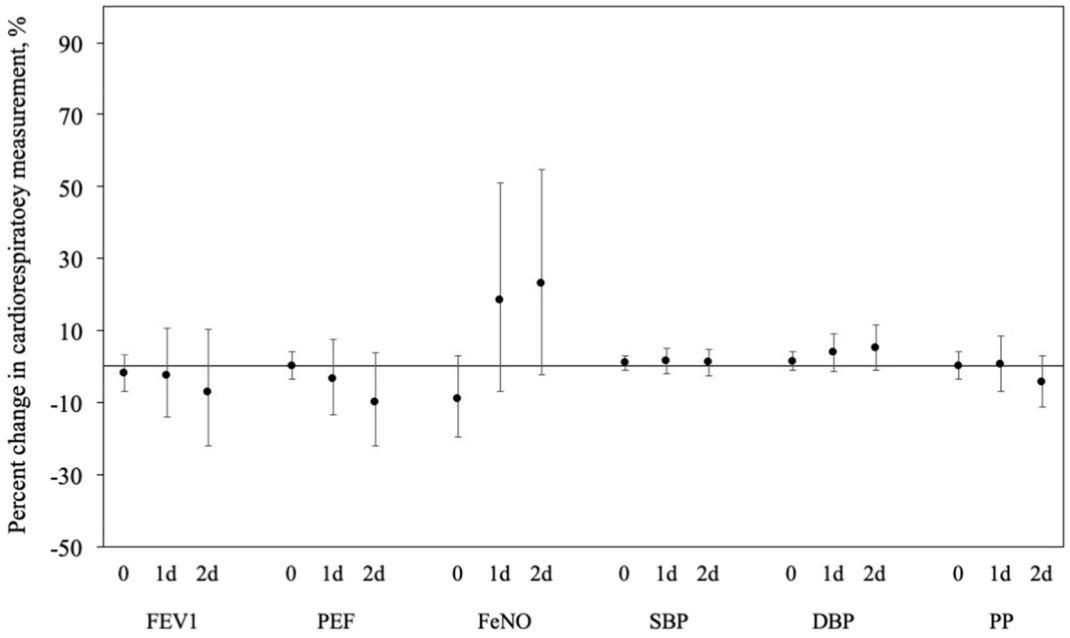

Fig. 4. Estimated percent changes with 95% confidence intervals in daily health measurements (including respiratory measurements and blood pressure) per IQR increase in ozone at different lagging days (lag 0, lag 1d and lag 2d).

offered important insights into the possible underlying biological mechanisms by which low-level indoor ozone may affect cardiovascular health in children, and indicated that more protective measures should be taken to alleviate the acute adverse effects of indoor ozone in this susceptible population.

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Declaration of Competing Interest

All authors declare they have no actual or potential conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2019.105021.

References

American Thoracic Society, European Respiratory Society, 2005. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am. J. Respir. Crit. Care Med. 171, 912–930.

Angelis, N., Spyratos, D., Domvri, K., Dimakopoulou, K., Samoli, E., Kalamaras, G., et al., 2017. Effect of ambient ozone exposure assessed by individual monitors on nasal function and exhaled no among school children in the area of Thessaloniki, Greece. J. Occup. Environ. Med. 59, 509–515.

Arjomandi, M., Wong, H., Donde, A., Frelinger, J., Dalton, S., Ching, W., et al., 2015. Exposure to medium and high ambient levels of ozone causes adverse systemic inflammatory and cardiac autonomic effects. Am. J. Physiol. Heart Circ. Physiol. 308, H1499–H1509.

Arjomandi, M., Balmes, J.R., Frampton, M.W., Bromberg, P., Rich, D.Q., Stark, P., et al., 2018. Respiratory responses to ozone exposure. Moses (the multicenter ozone study in older subjects). Am. J. Respir. Crit. Care Med. 197, 1319–1327.

Barath, S., Langrish, J.P., Lundback, M., Bosson, J.A., Goudie, C., Newby, D.E., et al., 2013. Short-term exposure to ozone does not impair vascular function or affect heart rate variability in healthy young men. Toxicol. Sci. 135, 292–299.

Baumann, C., Rakowski, U., Buchhorn, R., 2018. Omega-3 fatty acid supplementation improves heart rate variability in obese children. Int. J. Pediatr. 2018, 8789604.

Bell, M.L., McDermott, A., Zeger, S.L., Samet, J.M., Dominici, F., 2004. Ozone and short-term mortality in 95 us urban communities, 1987-2000. JAMA 292, 2372–2378.

Chen, P.C., Lai, Y.M., Chan, C.C., Hwang, J.S., Yang, C.Y., Wang, J.D., 1999. Short-term effect of ozone on the pulmonary function of children in primary school. Environ. Health Perspect. 107, 921–925.

Chen, C., Zhao, B., Weschler, C.J., 2012. Assessing the influence of indoor exposure to "outdoor ozone" on the relationship between ozone and short-term mortality in U.S. Communities. Environ. Health Perspect. 120, 235–240.

Chu, H., Hao, W., Cheng, Z., Huang, Y., Wang, S., Shang, J., et al., 2018. Black carbon particles and ozone-oxidized black carbon particles induced lung damage in mice through an interleukin-33 dependent pathway. Sci. Total Environ. 644, 217–228.

Cohen, A.J., Brauer, M., Burnett, R., Anderson, H.R., Frostad, J., Estep, K., et al., 2017. Estimates and 25-year trends of the global burden of disease attributable to ambient air pollution: an analysis of data from the global burden of diseases study 2015. Lancet 389, 1907–1918.

Day, D.B., Xiang, J., Mo, J., Li, F., Chung, M., Gong, J., et al., 2017. Association of ozone exposure with cardiorespiratory pathophysiologic mechanisms in healthy adults. JAMA Intern. Med. 177, 1344–1353.

Dai, Y., Qiu, H., Sun, S., Yang, Y., Lin, H., Tian, L., 2018. Age-dependent effect of ambient ozone on emergency asthma hospitalizations in Hong Kong. J. Allergy Clin. Immunol. 141, 1532–1534.

Devlin, R.B., Duncan, K.E., Jardim, M., Schmitt, M.T., Rappold, A.G., Diaz-Sanchez, D., 2012. Controlled exposure of healthy young volunteers to ozone causes cardiovascular effects. Circulation 126, 104–111.

Dominici, F., Peng, R.D., Bell, M.L., Pham, L., McDermott, A., Zeger, S.L., et al., 2006. Fine particulate air pollution and hospital admission for cardiovascular and respiratory diseases. JAMA 295, 1127–1134.

Electrophysiology TF, Task force of the european society of cardiology and the north american society of pacing and electrophysiology, 1996. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Circulation 93, 1043–1065.

Fournier, S., Taffé, P., Muller, O., 2013. Ischemic burden in ST elevation myocardial infaction and circadian rhythms. Circ. Res. 113, e42.

Gao, X., Xu, H., Shang, J., Yuan, L., Zhang, Y., Wang, L., et al., 2017. Ozonized carbon black induces mitochondrial dysfunction and DNA damage. Environ. Toxicol. 32, 944–955.

Gent, J.F., Triche, E.W., Holford, T.R., Belanger, K., Bracken, M.B., Beckett, W.S., et al., 2003. Association of low-level ozone and fine particles with respiratory symptoms in

children with asthma. JAMA 290, 1859–1867.
Gold, D.R., Damokosh, A.I., Pope 3rd, C.A., Dockery, D.W., McDonnell, W.F., Serrano, P., et al., 1999. Particulate and ozone pollutant effects on the respiratory function of children in southwest Mexico City. Epidemiology 10, 8–16.
Gong Jr., H., Wong, R., Sarma, R.J., Linn, W.S., Sullivan, E.D., Shamoo, D.A., et al., 1998. Cardiovascular effects of ozone exposure in human volunteers. Am. J. Respir. Crit. Care Med. 158, 538–546.
Huang, J., Pan, X., Guo, X., Li, G., 2018. Health impact of China's Air Pollution Prevention and Control Action Plan: an analysis of national air quality monitoring and mortality data. Lancet. Planet Health 2, e313–e323.
Jarrin, D.C., McGrath, J.J., Poirier, P., Seguin, L., Tremblay, R.E., Montplaisir, J.Y., et al., 2015. Short-term heart rate variability in a population-based sample of 10-year-old children. Pediatr. Cardiol. 36, 41–48.
Jerrett, M., Burnett, R.T., Pope 3rd, C.A., Ito, K., Thurston, G., Krewski, D., et al., 2009. Long-term ozone exposure and mortality. N. Engl. J. Med. 360, 1085–1095.
Jia, X., Song, X., Shima, M., Tamura, K., Deng, F., Guo, X., 2011. Acute effect of ambient ozone on heart rate variability in healthy elderly subjects. J. Expo. Sci. Environ. Epidemiol. 21, 541–547.
Kahle, J.J., Neas, L.M., Devlin, R.B., Case, M.W., Schmitt, M.T., Madden, M.C., et al., 2015. Interaction effects of temperature and ozone on lung function and markers of systemic inflammation, coagulation, and fibrinolysis: a crossover study of healthy young volunteers. Environ. Health Perspect. 123, 310–316.
Kan, H., Chen, R., Tong, S., 2012. Ambient air pollution, climate change, and population health in China. Environ. Int. 42, 10–19.
Kim, C.S., Alexis, N.E., Rappold, A.G., Kehrl, H., Hazucha, M.J., Lay, J.C., et al., 2011. Lung function and inflammatory responses in healthy young adults exposed to 0.06 ppm ozone for 6.6 hours. Am. J. Respir. Crit. Care Med. 183, 1215–1221.
Klepeis, N.E., Nelson, W.C., Ott, W.R., Robinson, J.P., Tsang, A.M., Switzer, P., et al., 2001. The national human activity pattern survey (nhaps): a resource for assessing exposure to environmental pollutants. J. Expo. Anal. Environ. Epidemiol. 11, 231–252.
Laszlo, G., 2006. Standardisation of lung function testing: helpful guidance from the ATS/ERS task force. Thorax 61, 744–746.
Lepeule, J., Laden, F., Dockery, D., Schwartz, J., 2012. Chronic exposure to fine particles and mortality: an extended follow-up of the Harvard six cities study from 1974 to 2009. Environ. Health Perspect. 120, 965–970.
Lin, S., Liu, X., Le, L.H., Hwang, S.A., 2008. Chronic exposure to ambient ozone and asthma hospital admissions among children. Environ. Health Perspect. 116, 1725–1730.
McConnell, R., Berhane, K., Gilliland, F., London, S.J., Islam, T., Gauderman, W.J., et al., 2002. Asthma in exercising children exposed to ozone: a cohort study. Lancet 359, 386–391.
Morgenstern, V., Zutavern, A., Cyrys, J., Brockow, I., Koletzko, S., Kramer, U., et al., 2008. Atopic diseases, allergic sensitization, and exposure to traffic-related air pollution in children. Am. J. Respir. Crit. Care Med. 177, 1331–1337.
Nickmilder, M., de Burbure, C., Carbonnelle, S., Dumont, X., Bernard, A., Derouane, A., 2007. Increase of exhaled nitric oxide in children exposed to low levels of ambient ozone. J. Toxicol. Environ. Health A 70, 270–274.
Pan, L., Wu, S., Li, H., Xu, J., Dong, W., Shan, J., et al., 2018. The short-term effects of indoor size-fractioned particulate matter and black carbon on cardiac autonomic function in COPD patients. Environ. Int. 112, 261–268.
Phadumdeo, V.M., Weinberg, S.H., 2018. Heart rate variability alters cardiac repolarization and electromechanical dynamics. J. Theor. Biol. 442, 31–43.
Raza, A., Bellander, T., Bero-Bedada, G., Dahlquist, M., Hollenberg, J., Jonsson, M., et al., 2014. Short-term effects of air pollution on out-of-hospital cardiac arrest in Stockholm. Eur. Heart J. 35, 861–868.
Rich, D.Q., Balmes, J.R., Frampton, M.W., Zareba, W., Stark, P., Arjomandi, M., et al., 2018. Cardiovascular function and ozone exposure: the Multicenter Ozone Study in oldEr Subjects (MOSES). Environ. Int. 119, 193–202.
Salonen, H., Salthammer, T., Morawska, L., 2018. Human exposure to ozone in school and office indoor environments. Environ. Int. 119, 503–514.
Sandrini, A., Taylor, D.R., Thomas, P.S., Yates, D.H., 2010. Fractional exhaled nitric oxide in asthma: an update. Respirology 15, 57–70.
Schelegle, E.S., Morales, C.A., Walby, W.F., Marion, S., Allen, R.P., 2009. 6.6-hour inhalation of ozone concentrations from 60 to 87 parts per billion in healthy humans. Am. J. Respir. Crit. Care Med. 180, 265–272.
Shah, A.S., Langrish, J.P., Nair, H., McAllister, D.A., Hunter, A.L., Donaldson, K., et al., 2013. Global association of air pollution and heart failure: a systematic review and meta-analysis. Lancet 382, 1039–1048.
U.S.EPA, 2015. National ambient air quality standards for ozone, final rule. Revision. In: Rules and Regulations. 80. Federal Register, pp. 65292–65468.
van Amsterdam, J.G., Nierkens, S., Vos, S.G., Opperhuizen, A., van Loveren, H., Steerenberg, P.A., 2000. Exhaled nitric oxide: a novel biomarker of adverse respiratory health effects in epidemiological studies. Arch. Environ. Health 55, 418–423.
van Biljon, A., McKune, A.J., DuBose, K.D., Kolanisi, U., Semple, S.J., 2018. Short-term high-intensity interval training is superior to moderate-intensity continuous training in improving cardiac autonomic function in children. Cardiology 141, 1–8.
Weschler, C.J., 2006. Ozone's impact on public health: contributions from indoor exposures to ozone and products of ozone-initiated chemistry. Environ. Health Perspect. 114, 1489–1496.
WHO, 2006. Air quality guidelines for particulate matter, ozone, nitrogen dioxide and sulfur dioxide. Global update 2005. Summary of risk assessment. http://wwwwhoint/phe/health_topics/outdoorair_aqg/en/indexhtml.
Wu, S., Deng, F., Niu, J., Huang, Q., Liu, Y., Guo, X., 2010. Association of heart rate variability in taxi drivers with marked changes in particulate air pollution in Beijing in 2008. Environ. Health Perspect. 118, 87–91.
Yin, P., Chen, R., Wang, L., Meng, X., Liu, C., Niu, Y., et al., 2017. Ambient ozone pollution and daily mortality: a nationwide study in 272 Chinese cities. Environ. Health Perspect. 125, 117006.